368-Pos Board B154

Thermodynamic Dissection of Estrogen Receptor-Promoter Interactions Reveals that Steroid Receptor Family Members Differentially Partition their Binding Energetics

Amie D. Moody, Michael T. Miura, Keith D. Connaghan, David L. Bain.
University of Colorado Denver, Aurora, CO, USA.

The quantitative principles responsible for steroid receptor-specific gene regulation are poorly understood. This is in part because most studies have measured only apparent binding affinities or used biochemical rather than biophysical approaches. We previously dissected the thermodynamics of human progesterone receptor (PR) isoform assembly at multisite promoter sequences. We found that the two isoforms maintain surprisingly weak dimerization energetics; however, the receptors can achieve full promoter occupancy via strong cooperative interactions. As a step towards assessing whether cooperative binding energetics are a common feature for all steroid receptors, we dissected the thermodynamics of human estrogen receptor-* (ER-*):promoter interactions under conditions identical to our work on PR. Analytical ultracentrifugation and quantitative footprint titrations demonstrate that ER-* and PR dimers maintain similar intrinsic binding affinities toward their respective response elements. This is to be expected noting the near identical structures of the ER and PR DNA binding domains. Unexpectedly, however, ER-* exhibits negligible cooperativity - orders of magnitude less than predicted based on our studies of PR. This raises the question of how ER-* generates full occupancy (and thus full function) at multisite promoters. Our results reveal that the decrease in ER-* cooperative free energy is exactly compensated for via an increase in dimerization free energy. Thus homologous transcription factors partition their promoter binding energetics differentially. We speculate that this serves as a mechanism to receptor-specific gene regulation: differences in assembly state and cooperative stabilization allow for preferential occupancy as a function of response element layout.

369-Pos Board B155

The Effects of Tension and Supercoiling on Protein-Mediated DNA Looping

Yue Ding[1], Carlo Manzo[2], David Dunlap[3], Laura Finzi[1].
[1]Emory University, Atlanta, GA, USA, [2]ICFO - The Institute of Photonic Sciences, Castelldefels (Barcelona), Spain, [3]Emory University School of Medicine, Atlanta, GA, USA.

Protein-mediated DNA looping is widely found as a topological regulatory mechanism in DNA transcription and other genetic process *in vivo*. The loop formation probability is mainly regulated by the loop size, protein concentration, supercoiling level of the genomic DNA, and local tension generated from the action of motor protein and enzymes. The supercoiling level varies at different times in cell cycle and likely at different locations in the genome, and a change of the local negative supercoiling level has been known to affect gene expression and regulation. However, little is known about the dynamics of how supercoiling and enzyme-generated tension control gene regulation by affecting DNA looping probability and stability.

We present single molecule measurements to study the effects of supercoiling and tension on a loop induced by the lambda repressor (CI protein) binding to two regions of specific sites in bacteriophage lambda DNA. The loop helps keeping the concentration of the repressor at the appropriate level to maintain the quiescent state, while guaranteeing efficient switching to virulence if necessary. Though even the lowest force prevents the wild type 2317 bp-long loop formation *in vitro*, the formation and breakdown of a 1051bp-long loop was observed under $<$1pN tension in unwound DNA at physiological CI concentrations. Furthermore, negative supercoiling stabilized the loop against increased tension, in agreement with our previous observation on a 400bp-long loop. The effect of DNA supercoiling on the formation and breakdown of different size loops is important for the quantitative understanding of the physiological role that DNA supercoiling and tension may have on loop-based gene regulation. Since the genome supercoiling level depends on the energy level and health status of a cell, our investigation sheds light on the dependence of some regulatory mechanisms on these two factors.

370-Pos Board B156

GalR Mediated Interactions Across the E. Coli Chromosome

Emilios K. Dimitriadis[1], Zhong Qian[2], Sankar Adhya[2].
[1]NIBIB, NIH, Bethesda, MD, USA, [2]NCI, NIH, Bethesda, MD, USA.

It has been reported that dimerized GalR could bind to 7 sites along the E.coli chromosome to regulate gene expression. Here, we label GalR at the C terminus with Venus fluorescence protein, for in-vivo localization in E.coli. In cells growing in stationary phase, 2 or 3 fluorescence spots per cell (55% and 31%) were detected regardless of the presence or absence of galactose. The spots indicate GalR oligomerization since tagged GalR monomers are below our detection limit. In log phase, however, we could not observe any similar spots. Furthermore, GalR mutations inhibiting tetramer formation abolished the spots also in stationary phase. We propose that GalR that binds to operator sites could oligomerize and facilitate long distance looping.

To examine this, we employed Atomic Force Microscopy (AFM) and Chromosome Conformation Capture (3C) analysis. By AFM we visualize GalR binding and consequent DNA looping. Analysis of the loop sizes identifies operator pairs bound via GalR oligomers and quantifies the cooperativity that brings distant operators together.

3C analysis suggests that DNA targets of GalR distributed around the chromosome are connected with one another mostly in stationary phase cells and that connection is lost in the log phase. Furthermore, we identified five additional potential binding sites of GalR along the investigated region of the E-coli chromosome.

371-Pos Board B157

Structural Alterations in the Nucleosome upon H3 Tail-Truncation Reveals a Crucial Role for the H2A C Terminal Docking Domain in Nucleosome Destabilization

Mithun Biswas[1], Joerg Langowski[2], Jeremy C. Smith[3].
[1]University of Heidelberg, Heidelberg, Germany, [2]German Cancer Research Center (DKFZ), Heidelberg, Germany, [3]University of Tennessee/ Oak Ridge National Laboratory Center for Molecular Biophysics, Oak Ridge, TN, USA.

The nucleosomal organization of eukaryotic chromatin offers a physical barrier to DNA access and also acts as a repository of epigenetic marks controlling chromosomal behavior during different periods of cell cycle. Post-translational modifications of histones play a key role in the regulation of gene access in eukaryotes. The majority of these modifications occur in the N-terminal extensions of the histone H3 in the form of methylation, acetylation or phosphorylation of amino acid residues. Here, we report on a total of 400 ns of all-atom molecular dynamics simulations of intact and tail-truncated nucleosomes and examine the effect of H3 tail truncation on nucleosome structure in atomic detail. During the intact nucleosome simulation one of the H3 tail domains showed propensity of alpha-helix formation. Upon truncation of the H3 tail containing the alpha-helical domain structural alterations occurred in the close by H2A(alpha)3 domain involving arginine residues and in the H2A C terminal docking domain suggesting a pathway for allosteric regulation of nucleosome stability. The relation between the present observations and corresponding experiments [1] is discussed.

Reference:
1. Ferreira et. al., Molecular and Cellular Biology 2007.

372-Pos Board B158

Specificity Profiles for DNA-Binding Proteins from Exhaustive In-Silico Screening

Daniel Seeliger[1], Floris P. Buelens[2], Maik Goette[3], de Groot L. Bert[2], **Helmut Grubmuller**[2].
[1]Boehringer Ingelheim Pharma GmbH, Ingelheim, Germany, [2]Max Planck Institute for Biophysical Chemistry, Goettingen, Germany, [3]German Institute for International Educational Research, Frankfurt, Germany.

DNA-binding regulates gene expression and, hence, is essential for cell function. A key prerequisite is the specific recognition of a particular nucleotide sequence. So far, identification of binding regions and positions has been approached rule-based, with rules extracted from experimental data. Here, we quantitatively assess the sequence-specific binding affinity of DNA-binding proteins by molecular dynamics-based alchemical free energy simulations. A computational framework was developed to automatically set up in silico screening assays and estimate free energy differences using two independent procedures, based on equilibrium and non-equlibrium transformation pathways [1], thus providing an internal cross check and error estimate. As an example, the binding specificity of a zinc-finger transcription factor to several sequences is calculated, and agreement with experimental data is shown [2]. As will be demonstrated, our approach allows to overcome the combinatorial problem of screening all possible sequence combinations, thus suggesting an efficient in silico screening strategy to obtain full specificity profiles for DNA-binding proteins.

[1] Goette M, Grubmuller H. Accuracy and convergence of free energy differences calculated from nonequilibrium switching processes. J. Comp. Chem. 30, 447-456 (2009)
[2] Seeliger D, Buelens FP, Goette M, de Groot BL, and Grubmueller H. Towards computional specificity screening of DNA-binding proteins. Nucl.Acids Res. July: 1-10 (2011)